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"Microstimulation of the Lumbosacral Spinal Cord"

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SUMMARY

We present the results from three animals (sp101-103). Two arrays containing 3 activated iridium microelectrodes each were implanted chronically into the S_1 or S_2 spinal cord of each animal. The activated iridium electrodes had geometric surface areas of approximately 2,000 μm^2. Twenty-eight days after implantation of the arrays, the cats were anesthetized with Propofol and one or two of the microelectrodes from each array were pulsed continuously for 12 hours on 2 successive days, using charge-balanced, cathodic-first controlled-current pulses, 150 μs/phase in duration. The pulse rate was 50 Hz and the pulse amplitude was 50 or 75 μA (7.5 or 11.2 nC/phase). The cats were sacrificed immediately after the 2nd day of stimulation.

Most of the neurons close to the pulsed electrode neurons appeared to be normal histologically. However, a few were undergoing chromatolysis, and although similar changes were observed adjacent to unpulsed electrodes, they seem to occur more frequently near the pulsed electrodes. Thus, we cannot exclude the possibility that the electrical stimulation might have contributed to this neural injury.

Variable amounts of tissue injury may have occurred during insertion of the electrodes, and/or during subsequent movement of the electrodes. Although the electrodes had been implanted at a fairly high velocity (~1m/sec), the histologic evaluation, and the videotapes taken at the time of implantation indicated that the spinal cord had dimpled and rotated slightly during the insertion process, and this undoubtably contributed to the tissue scarring and spongy changes in the fiber tracts. Also, some of the electrodes did not strike their intended target in the intermediolateral cell column. These difficulties probably are related to the fact that the sacral cord, as it tapers toward the stria terminalis, is very loosely suspended within the dural sack and the surrounding spinal roots. Also, the dorsal surface of the sacral cord is highly convex and does not easily accommodate an array inserted vertically. We have, therefore, decided to modify our array, to better accommodate the geometry of this difficult site. Six microelectrodes will extend from a single matrix button with a concave bottom (to fit the convexity of the dorsal surface of the sacral cord), and the arrays will

be further stabilized by a pair of long, slender iridium pins that will extend nearly completely through the cord.

INTRODUCTION

One objective of this contract is to develop electrode arrays suitable for chronic implantation into nuclei in the feline sacral spinal cord that control urogenital function. A second objective is to use these chronically-implanted arrays to determine protocols for safe and effective stimulation of the neurons in these nuclei. Our primary target is the intermediolateral cell column of the S_2 cord, which contains the preganglionic parasympathetic neurons that innervate the detrusor muscle of the urinary bladder. A secondary target is the neurons surrounding the central canal of the sacral cord, which may contain neurons that inhibit the motoneurons innervating the external urethral sphincter.

METHODS

Microelectrode arrays and surgical Procedures.

The microelectrode arrays contain 3 activated iridium microelectrodes, 1.4, 1.5 and 1.6 mm in length. The microelectrodes are 500 μm apart, and extend from an epoxy matrix 2 mm in length and approximately 0.75 mm in width. The microelectrodes themselves are 50 μm in diameter and are insulated with Epoxylite 6001 electrode varnish. The tips are fairly blunt (radius of curvature of 1.5 to 2 μm). The arrays have cables composed of 3 pure platinum wires, each 50 μm in diameter and insulated with Teflon. These cable are very flexible, and do not tend to dislodge the arrays after they have been implanted.

Three adult cats (sp101, sp102, sp103) were anesthetized with a mixture of nitrous oxide and Halothane. The spinal cord was exposed from the L_6 to S_3 root level with a standard dorsal laminectomy. The dorsal spinal process anterior to the laminectomy was secured with a vertebral clamp. In both cats, the target was the lateral cell column of the preganglionic parasympathetic nucleus at the S_2 level, whose neurons innervates the bladder detrusor muscle. The S_2 level of the spinal cord was located approximately by stimulating electrically the dermatome innervated by the S_2 root (the perigenital region) as a recording electrode was moved rostro-caudally over

the dorsal surface of the cord. The rostral-caudal position at which the maximum evoked response is recorded indicates the middle of the S_2 segment of the cord. A longitudinal incision was made through the dura at this level. The arachnoid was then dissected from the dorsal roots.

Two arrays, each containing 3 microelectrodes, were implanted into each of the 3 animals. They were implanted with the aid of a tool that allows the arrays to be inserted at a specified velocity. The instrument was mounted on the spinal apparatus, and oriented vertically (dorso-ventrally). The arrays were implanted 0.75 mm lateral to the cord's midline, at a velocity of approximately 1m/sec.

After implanting the array, the cables were sutured to the dura, rostral to the implant sites. The dura was closed very loosely with two 7-0 poly-filament sutures. The array cables were recurred back to the caudal end of the dural incision, and again sutured to the dura. A recording electrode was inserted through the dura, to lie along the ventral roots on the right side, and the reference recording electrode was placed in the epidural space. The loose dura closure was then covered with a patch of fascia resected from the para-spinal muscles. The para-spinal muscles were approximated with sutures, and the skin was closed with sutures.

Twenty-eight days after implanting the arrays, the cats were anesthetized with Propofol and 3 or 4 of the 6 microelectrodes were pulsed for 12 hours on each of two successive days. The stimulus waveform was cathodic-first, controlled-current pulses, 150 μs/phase in duration, at a rate of 50 Hz. The stimulus amplitude was 50 or 75μA. In cat sp101, 1 electrode was pulsed at 50 μA, 2 were pulsed at 75 μA, and 3 were not pulsed. In cat sp102, 3 electrodes were pulsed at 75 μA, and 3 electrodes were not pulsed. In cat sp103, 4 were pulsed at 50 μA, and 2 were not pulsed.

Immediately after the end of the second day of stimulation, the animals were deeply anesthetized with pentobarbital and perfused through the ascending aorta with 1 L of phosphate-buffered saline to remove blood, followed by 4 L of ½ strength Karnovsky's Fixative in 0.1 M sodium phosphate buffer at pH: 7.3 (½ K) (sp101, sp103), or with an immunofixative containing 4% formalin and 0.25% glutaraldehyde in 0.1 M sodium phosphate buffer (sp102). The sacral spinal cord was resected and the capsule

of connective tissue covering the elongated array matrices was removed with the arrays *in situ*. Spinal roots and nerves were identified to determine the exact level of the arrays. A segment of suture was then affixed to the surface of the matrix using rapid adhesion glue. After five minutes, the suture is used to draw the array straight up and out of the spinal cord. The tissue blocks were indexed with a small dot of tissue marking ink on their cut rostral surface, then embedded in paraffin. The paraffin-embedded tissue was cut at a thickness of 8 μm and stained with hematoxylin and eosin (H&E) or Nissl stains.

RESULTS

In all animals, the electrodes were positioned within the S_1 or S_2 segments of the cord (Figure 1). Autopsy examinations showed some variability in the positions of the electrode matrices on the dorsal surface of the dorsal columns. Subsequently, it was determined that in sp101 and sp103, some of the electrode tips of the rostral and caudal arrays were located within or adjacent to their intended target in the intermediolateral cell column of S_2.

Figure 2A-D show part of the track, and the site of the tip, of electrode #1 in the rostral array, from cat sp101. The electrode tip was in the dorsal horn, at the S_2 level, slightly dorsal and medial to the intended target in the intermediolateral cell column. Lateral to the site of the tip and shaft, there is a loosely-consolidated glial scar whose triangular shape suggests that it was caused by a lateral-to-medial (slashing) motion of the electrode's tip. The character of this injury, and the fact that the electrode was angled somewhat medially from its entry site on the dorsal surface, in spite of having been inserted vertically, suggests that the cord had rotated during insertion of the array. This electrode was pulsed at 75 μA and 50 Hz, for 12 hours on two successive days, well in excess of what would be required in a clinical protocol. However, neurons lateral and ventral to the tip site appear to be normal.

Figure 3A and B shows the track and tips of electrode #1 from the rostral array from cat sp 102. The electrode tip was in the caudal part of S_1, in the ventral part of the dorsal horn, and slightly medial and dorsal to the intermediolateral cell column. There

was relatively little mechanically-induced tissue injury. This electrode also was pulsed at 75 μA and 50 Hz, for 12 hours on two successive days. The neurons ventral to the electrode's tip site appear to be normal.

Figures 4A and B show the site of the tip of the middle electrode from the same array. The tip was quite close to the intermediolateral cell column. Again, there was relatively little mechanically-induced injury, and the neuropil surrounding the track and tip site appears quite normal, although the neuropil appears to have been distended and displaced ventrally (a curiously persistent phenomenon that we have also noted in the cochlear nucleus, several months after electrode implantation). This electrode was also pulsed at 75 μA. Neurons within 40 μm of the site of the tip appear to be normal.

Figures 5A and B show the track and the tip site of the middle electrode #2 from the rostral array from cat sp103. The electrode's tip was in the intermediolateral cell column, and close to the intended target in S_2. However, this placement was serendipitous, since the electrode had penetrated the dorsal column a point medial to the intended target, and then had angled sharply laterally, apparently as the cord dimpled and rotated during the insertion process. There is some gliotic scarring in the neuropil medial to the track, and other evidence of slashing injury. This electrode was also pulsed at 75 μA, and the neurons close to the tip appear to be either normal, or showed evidence of chromatolysis.

Figures 6A and B show the track and tip site of electrode #1 in the caudal electrode array from the same cat (sp103). The electrode's tip is close to the central canal, in the region where inhibitory neuron implicated in urogenital function have been located. The electrode inflicted relatively little mechanical injury and the neuropil surrounding the tip site appears relatively normal. This microelectrode was pulsed at 50 μA on 2 successive days. Most of the neurons close to the tip appear normal. However, a few are undergoing chromatolysis. Similar neuronal chromatolysis was observed in neurons adjacent to unpulsed electrodes, or distant from the shafts and tips (Figure 7). These cellular changes probably are related to the mechanically-induced tissue injury; neurons will undergo chromatolysis when their axons are severed. However, there was relatively little evidence of other mechanically-induced tissue injury near the tip of the

electrode shown in Figure 6B, and we cannot exclude the possibility that the electrical stimulation may have contributed to the chromatolysis.

Table I summarizes the observations from the three animals. The major histologic changes near the electrode tracks included fibrotic scars in the gray matter and spongy changes in the fiber tracts (white matter), both apparently associated with varying amount of slashing injury by the electrodes. Varying amounts of neovascularization was present adjacent to the electrode tracks. Few, if any microhemorrhages were seen. Little or no vasculitis and minimal deposits of hemosiderin pigments were observed adjacent to the electrode tracks. A few leukocytes, mainly lymphocytes and mononuclear cells, were observed near the electrode tips and shafts. However, there was no apparent association between electrode pulsing and the presence of leukocytes and relatively few of these inflammatory cells were present.

A few neurons close to (and also a few distant from) the electrode shafts and tips were undergoing chromatolytic changes. Although most neurons near the tips of the pulsed electrodes appeared to be normal, and chromatolytic neurons were also seen near some of the unpulsed electrodes, we cannot exclude the possibility that the electrical stimulation may have contributed to the chromatolysis.

TABLE I

ANIMAL #	ELECTRODE #	STIMULUS CURRENT (μA)	ELECTRODE TIP WITHIN OR NEAR THE LIM CELL COLUMN	SLASHING INJURY PRESENT	LEUKOCYTES NEAR TIP (T) OR CAPSULE (C)	NEURONS UNDERGOING CHROMATOLYSIS (Number of neurons in one histologic section)
SP-101	Rostral array electrode #1	75	Yes	Yes	C	0
	R. E #2	50	Yes	Yes	C	0
	R. E #3	none	Yes	Yes	C	1 (shaft)
	Caudal array electrode #1	none	Yes	No	T	0
	C. E #2	none	Yes	Yes	C	1 (d-shaft)
	C. E #3	75	Yes	Yes	C	0
SP-102	R. E #1	75	Yes	No	None	0
	R. E #2	75	Yes	No	None	0
	R. E #3	none	Yes	No	C (few)	0
	C. E #1	none	No*	--	--	0
	C. E #2	none	No*	--	--	0
	C. E #3	75	No*	--	--	0
SP-103	R. E #1	none	yes	Yes	C	3 (shaft)
	R. E #2	50	Yes	Yes	T	5 (tip/shaft)
	R. E #3	50	Yes	Yes	C (few)	4 (shaft)
	C. E #1	50	No[&]	No	T	4(shaft)
	C. E #2	50	No[&]	No	T (few)	2(shaft)
	C. E #3	none	No[&]	No	T	2(d-shaft)

*Electrode tip within lateral funiculus (white column)

[&] Electrode tip near central canal

d-shaft = chromatolysis observed within neurons greater than 200 μm from the electrode shaft or tip.

DISCUSSION

In the present series, the severity of mechanically-induced tissue injury, while by no means insignificant, was sufficiently controlled so the effects of the electrical stimulation could be assessed on neurons very close (20-50 μm) to the chronically-implanted intraspinal microelectrodes. The stimulus amplitude was 50 or 75 μA (7.5 or 11.2 nC/phase) and the stimulus frequency was 50 Hz. The stimulation was administered continuously for 12 hours on two successive days. Our results indicate that this regimen probably does not induce histologically-detectable changes in the neurons of the intermediate gray column of the feline sacral spinal cord. However, a few neurons close to the pulsed microelectrodes were undergoing chromatolysis, and we cannot exclude the possibility that the electrical stimulation may have contributed to these changes. There was no detectable correlation between electrode pulsing and lymphocyte accumulation near the electrode tips. Such a correlation has been noted in studies of electrical stimulation in the feline cerebral cortex (Yuen et al., 1998). However, in the cerebral cortex, the aggregates of lymphocytes are markedly less dense when the stimulation is extended beyond one day. This indicates that the aggregation of lymphocytes around pulsed microelectrodes is a transient phenomenon. This premise is supported by the present studies, in which we observed a paucity of lymphocytes within the tissue in which the electrodes were pulsed for 12 hours on two successive days and sacrificed immediately after the 2nd day of stimulation.

In the present series, the arrays of three microelectrodes, 1.4 to 1.6 mm in length, were implanted from the dorsal surface of the cord, with the aid of a rapid-insertion device. Even when the arrays are implanted at about 1 m/sec, there is evidence that the loosely-suspended sacral cord had dimpled and rotated during electrode insertion. Many electrodes did not traverse a dorsal-to-ventral trajectory, although they had been inserted vertically. The rotation apparently was responsible for the significant tissue injury seen in all 3 animals, and it also made it very difficult to implant the electrode into their intended target within the intermediolateral cell column. There are also indications

that the array in the S_2 segment of cat sp102 had dislodged after implantation, and then had re-implanted itself into the cord, well lateral to its intended target. All three electrodes in this array were situated within the extreme lateral margin of the lateral white column, and the tips were barely within the spinal cord. This instability probably can be attributed to the fact that the dorsal surface of the sacral cord is very convex, particularly at the S_2 level, and is not a good platform for an array with a planar lower surface, when the array is inserted in a dorsoventral direction. However, we have shown that single-shaft electrodes are very unstable in the sacral cord, and a multi-electrodes array must be inserted vertically from the cord's dorsal surface, since the risk of injury to the dorsal roots excludes inserting it lateral to the dorsal root entry zone. We have, therefore, decided to modify our microelectrode array. Six microelectrodes will extend from a single matrix button, whose concave lower surface will straddle the convex dorsal surface of the lower sacral cord. The array will be positioned over the midline of the dorsal columns, and inserted vertically. Three microelectrodes on each side of the array will extend down into each intermediolateral cell column. In addition, the array will contain 2 long (3 mm) stabilizing pins, which will extend nearly to the ventral surface of the sacral cord, and will help to anchor the array after implantation. This is similar to a design that we have used in the cerebral cortex, and in a microelectrode array intended for implantation into the human cochlear nucleus. Since the array will be centered over the dorsal columns, the cord should not rotate during insertion, although it may be displaced vertically. To counter the later, the array will have to be inserted at a fairly high velocity. The first of these novel arrays will be implanted during the next contract quarter.

LITERATURE CITED

Yuen TGH, Agnew WF, McCreery DB, Bullara LA (1998). Accumulation of lymphocytes elicited by microstimulation of the cat's cerebral cortex. Abstr. Soc. for Neuroscience 24:659.

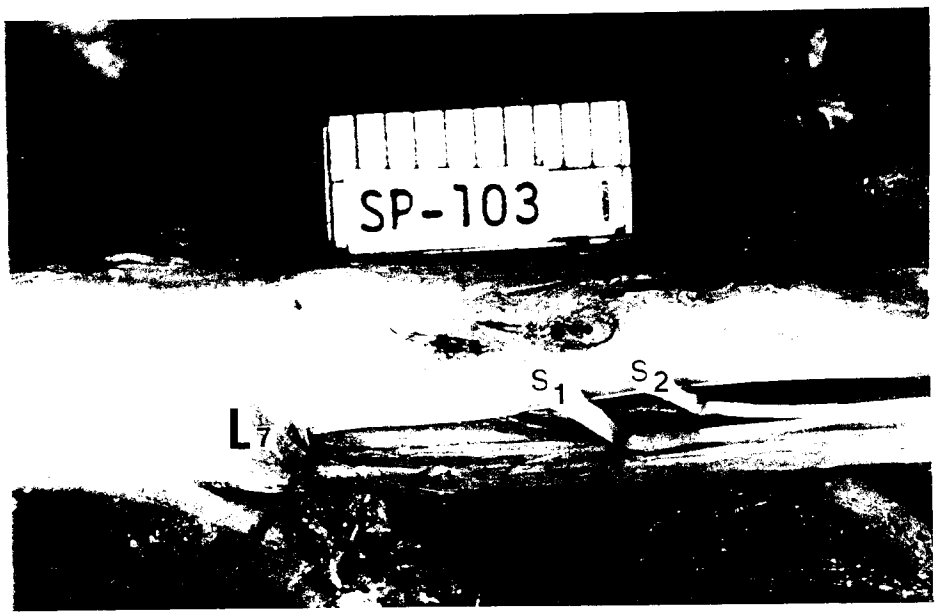

Figure 1. sp103. Two microelectrode arrays are shown implanted within the dorsal sacral spinal cord. Note the arrays are within the S_1 or S_2 regions. L_7 is indicated for reference.

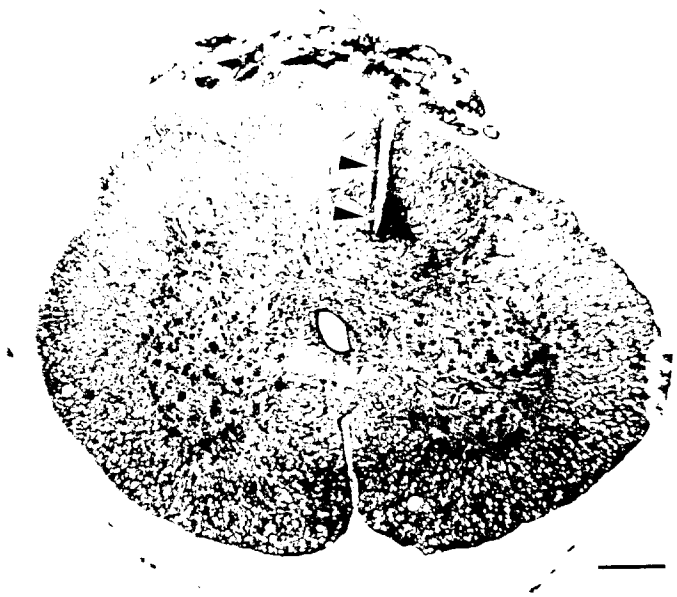

Figure 2A. sp101, rostral array, pulsed electrode #1. Panoramic view of the electrode track within the dorsal horn (arrowheads). Nissl stain. Bar = 500 µm.

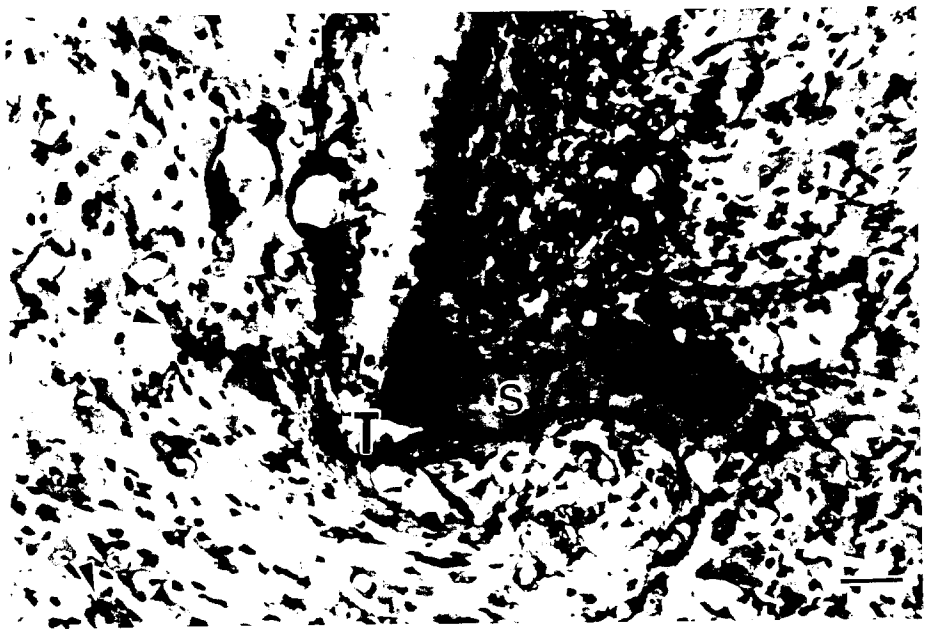

Figure 2B. sp101, rostral array, pulsed electrode #1. Higher magnification shows a gliotic scar (S) near the tip (T) of this electrode. Local neurons (arrowheads) appear normal. Nissl stain. Bar = 50 µm.

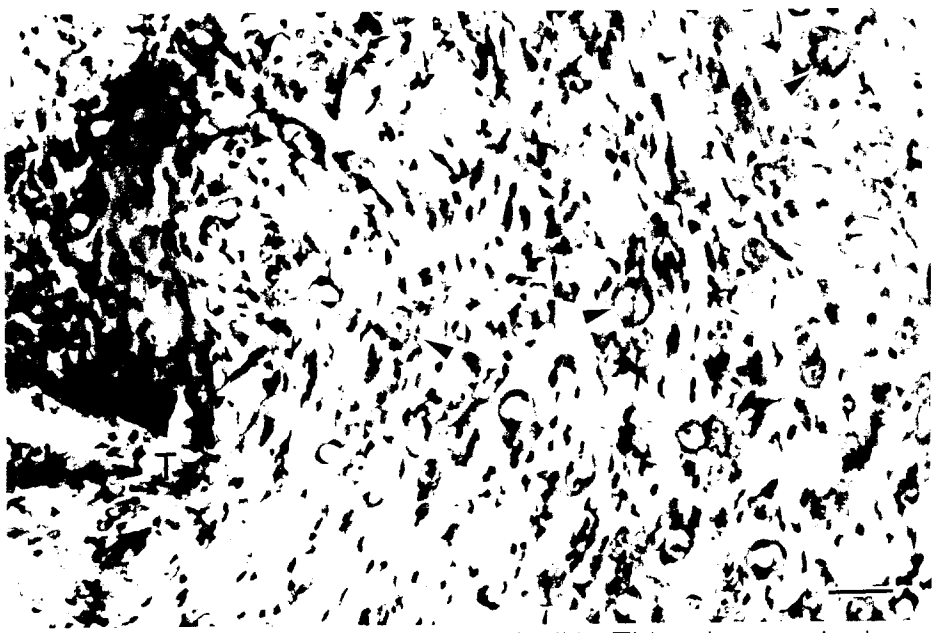

Figure 2C. sp101, rostral array, pulsed electrode #1. This micrograph shows another region adjacent to the tip of the electrode (T). Note the normal appearance of the neurons (arrowheads) adjacent to and distant from, the electrode tip.
Nissl stain. Bar = 50 µm.

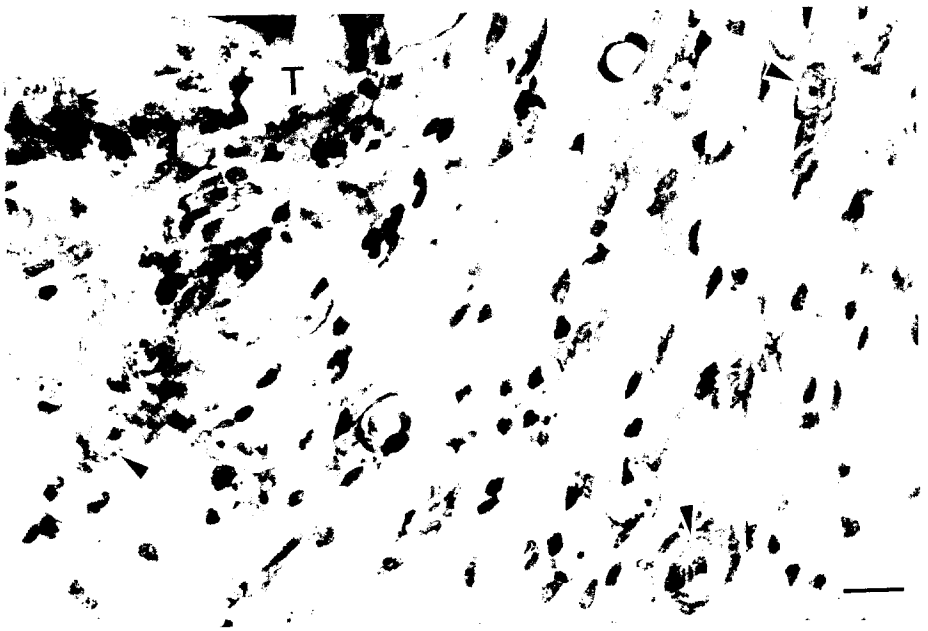

Figure 2D. sp101, rostral array, pulsed electrode #1. Note the higher magnification images of normal-appearing neurons (arrowheads) near the electrode tip (T). Nissl stain. Bar = 25 µm.

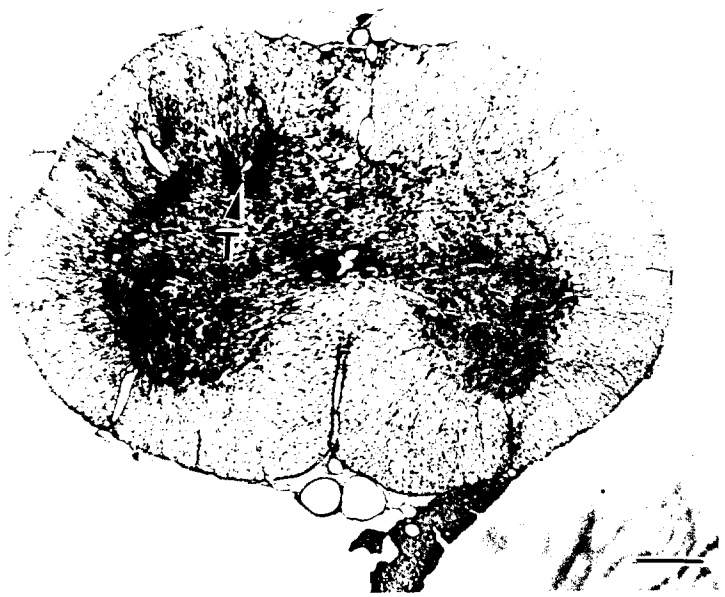

Figure 3A. sp102, rostral array, pulsed electrode #1. Note the position of the electrode tip (T arrowhead) in this panoramic view of the spinal cord. Nissl stain. Bar = 500 µm.

Figure 3B. sp102, rostral array, pulsed electrode #1. Besides reactive astrocytes (arrowheads), little tissue injury is noted. Neurons (arrows) adjacent to the electrode tip (T) and distant to it appear normal. Nissl stain. Bar = 50 µm.

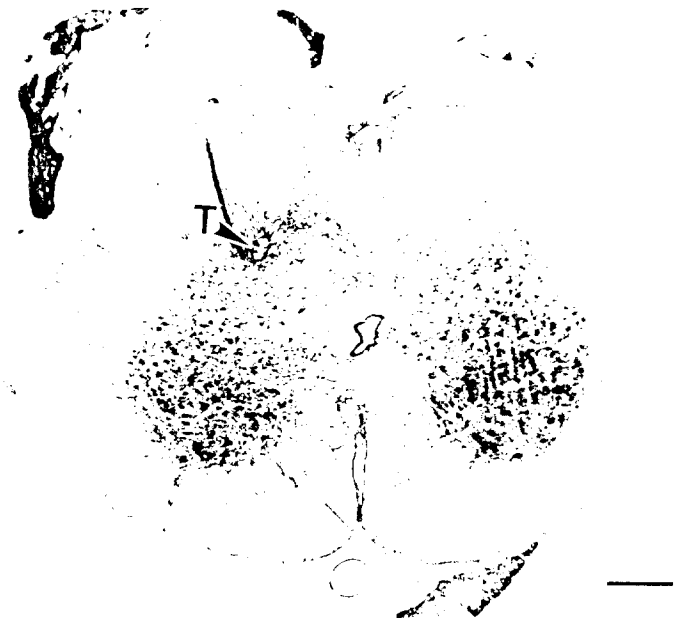

Figure 4A. sp102, rostral array, pulsed electrode #2. Note the laterally-directed course of this electrode(*). The electrode tip (T arrowhead) is shown. Nissl stain.
Bar = 500 µm

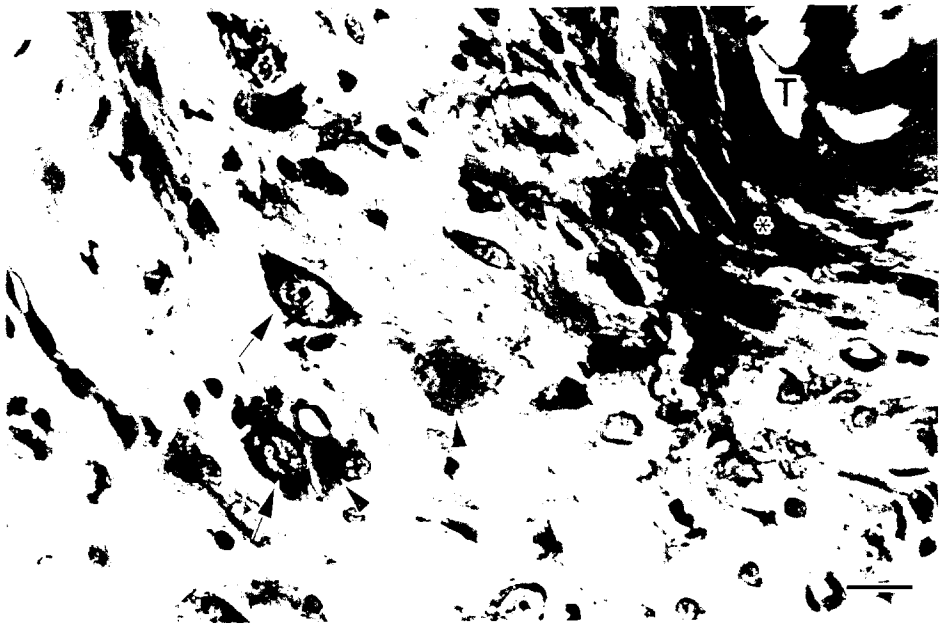

Figure 4B. sp102, rostral array, pulsed electrode #2. Higher magnification adjacent to the tip (T) of this electrode shows reactive astrocytes (arrowheads), and the normal appearance of local neurons (arrows). A thick fibrotic electrode sheath is also shown (*). Nissi stain. Bar = 25 µm.

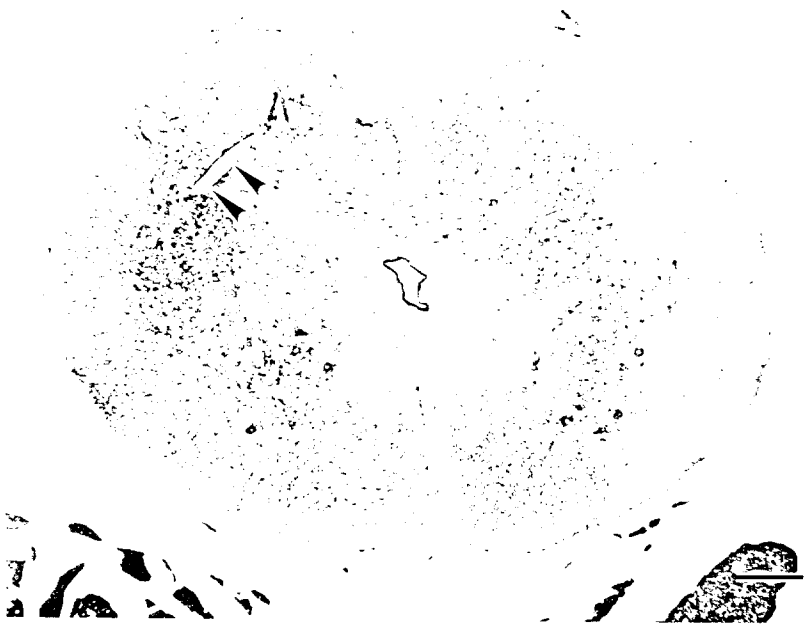

Figure 5A. sp103, rostral array, pulsed electrode #2. The electrode (arrowhead) is directed laterally. The array matrix has depressed the dorsal surface of the cord. H & E stain. Bar = 500 µm.

Figure 5B. sp103. rostral array. pulsed electrode #2. In the vicinity of the electrode tip (T) and some distance for the shaft are five neurons undergoing chromatolysis (arrowheads). Other neurons appear normal (*). Nissl stain. Bar = 50 µm.

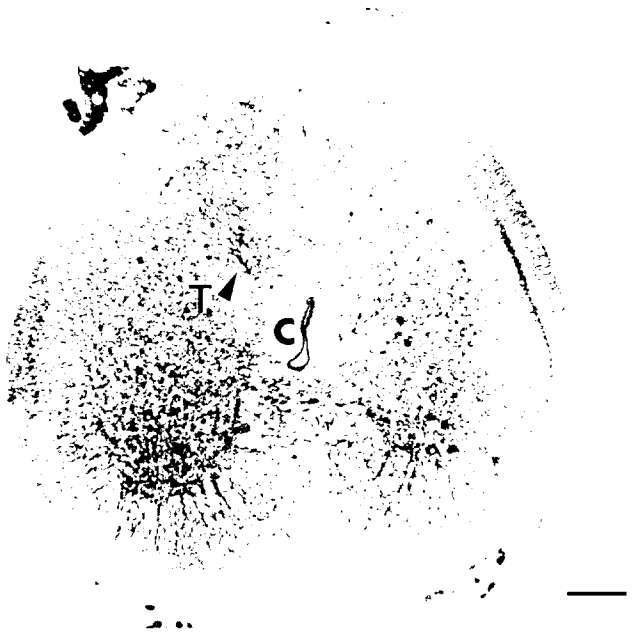

Figure 6A. sp103. caudal array. pulsed electrode #1. Panoramic view shows dimpling of the cord's dorsal surface and the electrode tip (T arrowhead) within the vicinity of the central canal (C). Nissl stain. Bar = 500 µm.

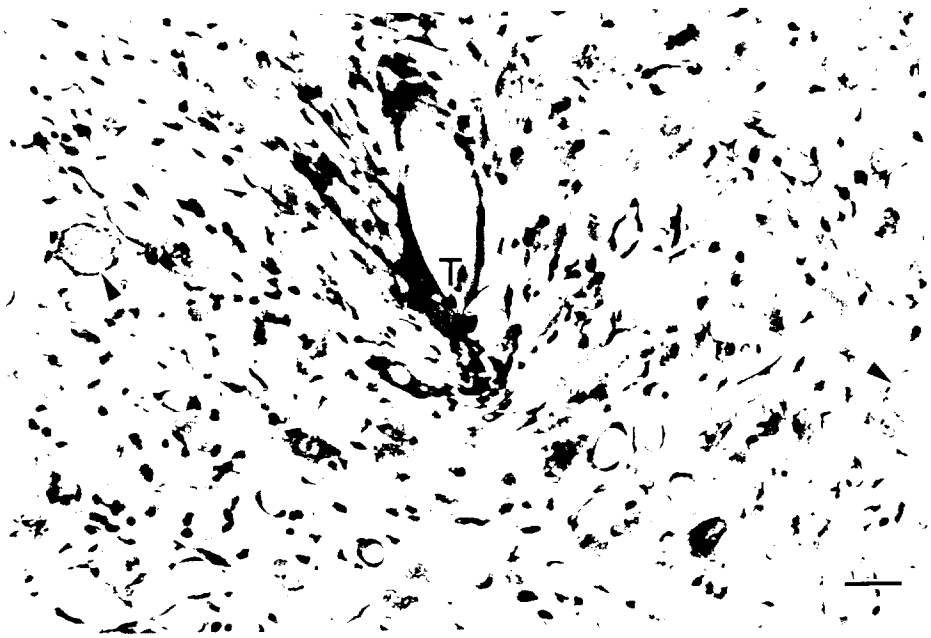

Figure 6B. sp103, caudal array, pulsed electrode #1. Note the minimal tissue injury near the electrode tip (T). Two neurons are undergoing chromatolysis (arrowheads). Nissl stain. Bar = 50 µm.

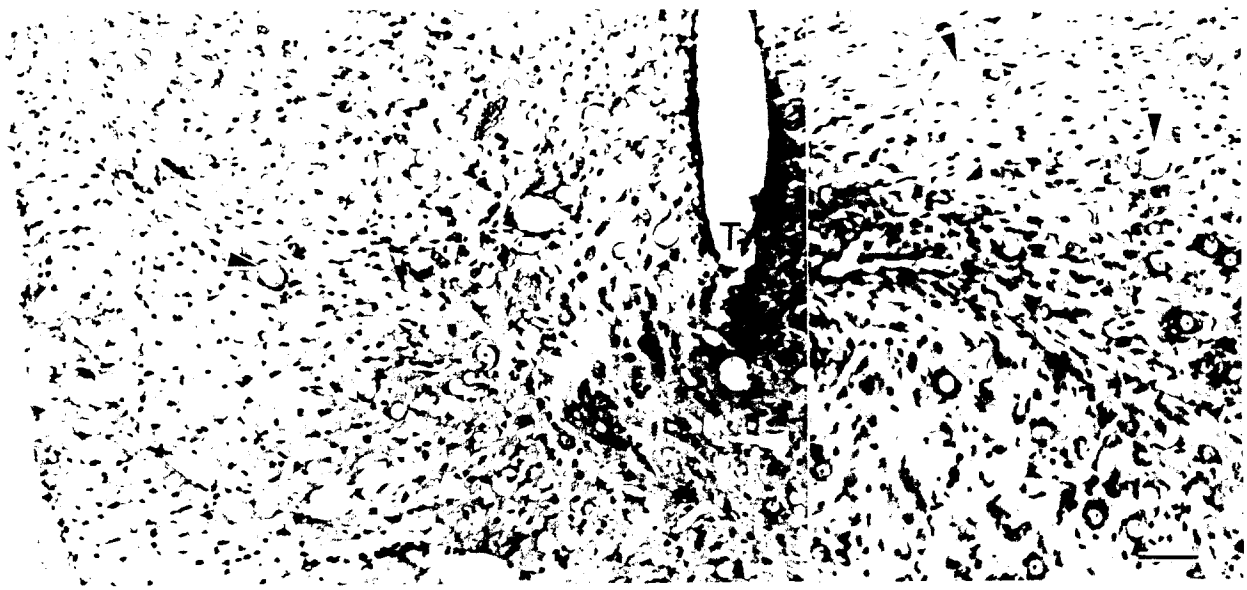

Figure 7. sp103, rostral array, unpulsed electrode #1. The electrode track and tip (T) are shown. Three neurons are undergoing chromatolytic (arrowheads). Nissl stain. Bar = 100 µm.